

2X Thermo-Start™ PCR Master Mix

Description: Thermo-Start™ PCR Master Mix contains in a single vial, all the components to perform a rapid and reproducible Hot Start PCR. This Master Mix includes the Thermo-Start™ *Taq* DNA Polymerase which is a chemically modified version of ThermoPrime. This results in a completely inactive polymerase at room temperature, preventing the formation and subsequent amplification of non-specific products. The enzyme requires an **activation step at 95°C for 15 minutes.**

Enzyme Source: *Thermus aquaticus*

Associated Activities: Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

Storage Conditions: Store Thermo-Start™ *Taq* DNA Polymerase at -20°C, in a constant temperature freezer for up to 12 months. Shipped on ice within the UK and on dry ice for international and within the US.

Kit Contents

Vial	Pack Size (cap color)	
	A	B
2X Thermo-Start PCR Master Mix	2 x 1ml (purple)	20 x 1ml (purple)

Master Mix: Each vial contains 1.0ml of a 2X working concentration PCR Master Mix sufficient for 80 x 25µl reactions. The final reaction 1X contains:

0.625 units	Thermo-Start™ <i>Taq</i> DNA Polymerase
1X	Thermo-Start® reaction buffer
0.2mM	each of dATP, dCTP, dGTP and dTTP
(see table 1)	MgCl ₂

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Example of Protocol:

Mix and spin down the solutions prior to use

	Volume	Final Concentration 1X
2X Thermo-Start PCR Master Mix	12.5µl	1X
Primer forward (10µM each)	1.25µl*	0.5µM*
Primer reverse (10µM each)	1.25µl*	0.5µM*
Water (PCR Grade)	variable	
DNA Template	0.5 - 10µl	0.5 - 125ng
Total Volume	25 µl	

*Scale up or down the volume and concentration as appropriate

Note: These recommendations are intended as basic guidelines. Magnesium chloride concentration and amount of enzyme should be optimized according to template and primer combination.

Example of Program:

	Temp.	Time	Number of cycle
Initial Denaturation	95°C	15 min	1 cycle
Denaturation	95°C	20 sec	30 to 40 cycles
Annealing	50-65°C	30 sec	
Extension **	72°C	60 sec	
Final Extension	72°C	5 min	1 cycle

** Increase length of time in proportion to size of amplicon, *Taq* DNA Polymerase extends at approximately 1000 bp/min.

Incremental Activation:

For extra stringency, the enzyme can be activated gradually during the PCR in a series of steps. The initial activation step is replaced by longer (2 minutes) denaturation steps for the first 7–8 cycles of the reaction.

Analysis Conditions:	25mM	TAPS, pH 9.3 (at 25°C)
	50mM	[tris-(hydroxymethyl)-methyl-amino-propane sulfonic acid, sodium salt]
	2mM	KCl
	1mM	MgCl ₂
	200µM	β-mercaptoethanol
	100µM	of each: dATP, dGTP, dTTP
	1.25µg/µl	[α ³² P]-dCTP (0.05 to 0.1 Ci/mmol) activated salmon sperm DNA

Water added to a total volume of 50µl. Incubated at 74°C for 10 minutes. The enzyme is first treated with a 15 minute activation step at 95°C. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

Ordering Information:

AB-0938/DC/MM/A	2X Thermo-Start PCR Master Mix	160 x 25µl rxns
AB-0938/DC/MM/B	2X Thermo-Start PCR Master Mix	1600 x 25µl rxns

Note: MM denotes MgCl₂ concentration (see table 1).

Table 1

Cat. No.	MgCl ₂ final concentration
AB-0938/DC/15	1.5mM
AB-0938/DC/20	2.0mM
AB-0938/DC/25	2.5mM
AB-0938/DC/30	3.0mM
AB-0938/DC/35	3.5mM
AB-0938/DC/40	4.0mM

Troubleshooting

For troubleshooting, see www.abgene.com/troubleshoot.asp or contact Thermo Fisher Scientific (ABgene) TechSupport at abgene.techsupport@thermofisher.com

UK TechSupport, call +44 (0) 1372 840 410

For all other regions, please contact your local Thermo Fisher Scientific (ABgene) office / distributor.

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